

Amendments to the Specification:

Please replace the original paper copy of the Sequence Listing with the substitute paper copy of the Sequence Listing filed herewith.

At page 1, line 1, please delete subheading:

~~DESCRIPTION~~

Please amend the title to read as:

METHODS FOR PRODUCING ANTIBODIES

Insert the following paragraph on page 1, line 3, after the title:

Claim of Priority

This application is the National Stage of International Application No. PCT/JP03/07071, filed June 4, 2003, which claims the benefit of Japanese Patent Application Serial Nos. 2002-164834, filed on June 5, 2002, and 2002-180351, filed on June 20, 2002, the entire contents of which are hereby incorporated by reference.

Please replace the paragraph beginning at page 7, line 25, with the following amended paragraph:

Of these methods, transgenic non-human animals which preserve a gene encoding an immunotolerance antigen in an expressible state are preferred as the non-human animals comprising immunotolerance of the present invention. The transgenic animals comprise in their body an immunotolerance antigen that was originally an exogenous protein prior to the maturation of immune functions. Therefore, it is highly possible that the immune functions of the transgenic animals recognize the immunotolerance antigen as being completely endogenous. Thus, the use of such transgenic non-human animals is advantageous in inducing immunotolerance in the present invention. The transgenic animals, into which immunotolerance

antigens are introduced, produce few antibodies to immunotolerance antigens, as shown in Example[[s]] 8.

Please replace the paragraph beginning at page 17, line 6, with the following amended paragraph:

More specifically, transgenic mice can be prepared, for example, by the method in Example[[s]] 2.

Please replace the paragraph beginning at page 24, line 7, with the following amended paragraph:

Fig. 3 shows the structure of the pCAG-gp64 vector constructed in Example[[s]] 1.

Please replace the paragraph beginning at page 25, line 6 with the following amended paragraph:

The PCR reaction solution composition was 5 µl of x10 ExTaq buffer, 4 µl of dNTP supplied with ExTaq, 1 µl of 10 µmol/l 64F1 primer, 1µl of 10 µmol/l 64R1 primer, 1µl of 500 pg/µl pBac-N-blue, 0.5 µl of 5 units/µl ExTaq, and 37.5 µl of deionized water (DIW). PCR was carried out for:

5 minutes at 94°C;

25 cycles of “15 seconds at 94°C, 30 seconds at 57°C, and 30 seconds at 72°C”;

7 minutes at 72°C; and

4°C forever.

Please replace the paragraph beginning at page 25, line 15, with the following amended paragraph:

The amplified band was subcloned into pGEM-Teasy, and then transformed *E. coli* DH5α cells. After performing colony PCR using T7 and SP6 primers, the nucleotide sequence of clones confirmed to comprise the insert was analyzed with the ~~ABI Prism377 DNA sequencer~~

~~and the BigDye Cycle Sequence kit~~ ABI Prism® 377 DNA sequencer (Applied Biosystems) and the BigDye® Terminator Cycle Sequencing kit (Applied Biosystems), in combination with the T7 primer or the SP6 primer. As a result, clones comprising the subject gene were confirmed. A fragment comprising the gp64 gene and confirmed to comprise no mutations in its nucleotide sequence was isolated from the clones by EcoRI digestion, and then inserted into an EcoRI-digested pCAGGS1. The resulting vector was used to transform *E. coli* DH5 $\alpha$  cells. Cells comprising the clone as designed were incubated in 250 ml of LB medium at 37°C overnight, and purified by using the Endofree MAXI kit (QIAGEN) to obtain 581.6  $\mu$ g of plasmid.

Please replace the paragraph beginning at page 25, line 36, with the following amended paragraph:

The mouse pronuclear eggs to be injected with the DNA fragment were collected as follows: Specifically, BALB/c series female mice (Nippon CLEA) were induced to superovulate by intraperitoneal administration of 5 international units (i.u) of pregnant mare serum gonadotrophin (PMSG), followed by intraperitoneal administration of 5 i.u of human chorionic gonadotrophin (hCG) 48 hours later. These female mice were mated with male mice of the same lineage. The morning after mating, the oviducts of female mice that were confirmed to have a vaginal plug were perfused to recover pronuclear eggs.

Please replace the paragraph beginning at page 26, line 8, with the following amended paragraph:

The DNA fragments were injected into the pronuclear eggs with a micromanipulator (Experimental Medicine (Jikken Igaku) suppl., [( )] The latest technologies in gene targeting (gene targeting no saishin gijyutu) (Yodosha), 190-207, 2000). The DNA fragments were injected into 373 embryos of BALB/c mice. On the next day, 216 embryos that had developed to the two-cell stage were transplanted into the oviducts of recipient female mice, which were in the first day of pseudopregnancy, at a density of around ten embryos per oviduct (i.e. around 20 embryos per mouse).

Please replace the Table 2 on page 27 with the following amended Table 2:

Table 2

Line No.	Date of birth	Sex	Copy Number of the introduced gene	Offspring obtained (date of birth, total offspring, and Tgm)			Notes
30	010709	Male	More than 10 copies	010926	Female 3, Male 1	Female 3	No offspring were obtained after the first delivery. Testes are small and sperm are not observed.
31	010709	Male	2 to 3 copies	010927	Female 3, Male 5	0	Mosaic for gene transfer
				011022	Male 2	0	
				011108	Female 4, Male 6	0	
34	010709	Male	2 to 3 copies	No fertility properties	-	-	Testes are small and sperm are not observed.
46	010821	Male	2 to 3 copies	No fertility properties	-	-	Testes are small and sperm are not observed.

Please replace the Table 3 on page 29 with the following amended Table 3:

Table 3

Sex	Individual Number	Number of Deliveries	Offspring (Non-Tgm)	Offspring (Tgm)
Female	1	2	Female 3, Male 1	Female 1, Male 6
Female	2	2	Female 4, Male 3	Female 2, Male 1
Female	3	2	Female 2, Male 4	Female 2, Male 2

Please replace the paragraph beginning at page 31, line 5, with the following amended paragraph:

PepT1 expressing budded baculovirus (PepT1-BV) (1 µg/lane) was subjected to SDS-PAGE analysis on 12% gel under reducing conditions. After the electrophoresis, proteins were electroblotted onto a polyvinylidene difluoride (PVDF) membrane. This membrane was reacted

with 1,000 fold-diluted serum samples, sequentially washed, and then reacted with a 1,000 fold-diluted Biotin-Anti-Mouse IgG( $\gamma$ ) (Zymed) and Streptavidin-Alkaline Phosphatase (Zymed). An alkaline phosphatase staining kit (Nakarai Tesque) was used for staining. A positive control antibody for detecting gp64 was purchased from NOVAGEN.

Please replace the paragraph beginning at page 31, line 21, with the following amended paragraph (note that the only change is to correct the numbering of the example):

[Example [[8]] 2] Production of anti-PepT1 antibodies by gp64 Tgm

Please replace the paragraph beginning at page 32, line 16, with the following amended paragraph:

For example, an exogenous gene expression system, known as the baculovirus expression system, is useful as a tool for obtaining recombinant proteins easily and in large quantities. In particular, when applied to membrane proteins, the baculovirus expression system is excellent in that the membrane proteins are obtainable with other viral envelope proteins in a state that maintains their structure. However, this expression system is also problematic in that, when using this expression product as the immunogen, gp64 acts as a background ~~protein~~ antigen and interferes with the acquisition of antibodies against a target ~~protein~~ antigen.